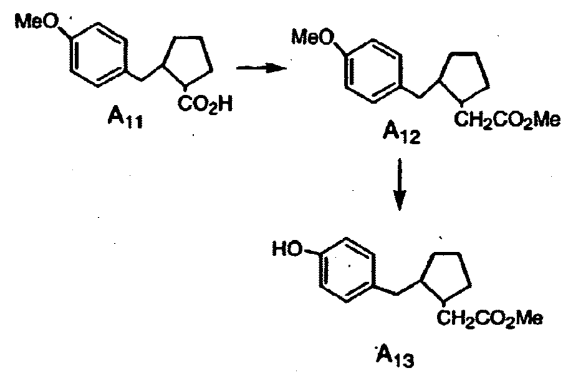
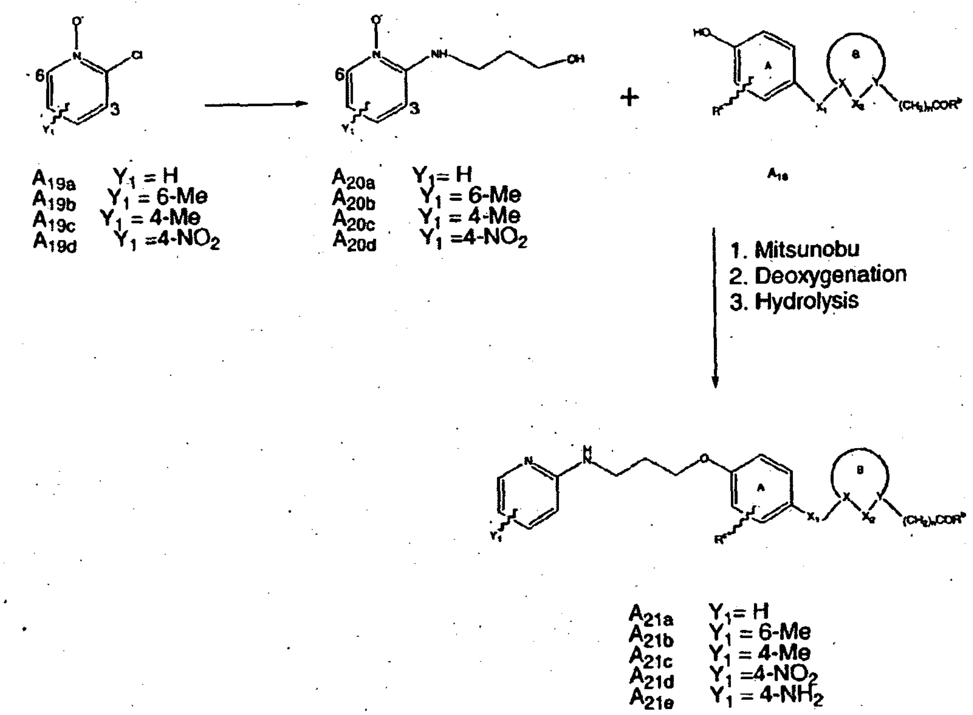
SCHEME 5



SCHEME 5

Compounds of FORMULA I, wherein X_1 is CH_2 are prepared starting with commercially accessible intermediate A_{11}. Reduction of the carboxylic acid functionality in A_{11} with e g; diborane or lithium aluminum hydride gives the hydroxymethyl derivative which may be elaborated to CH_2CO_2R functionality using the methodology elaborated in EXAMPLE 1. Demethylation of the intermediate with a boron trihalide such as boron tribromide, boron trichloride gives the demethylated intermediates A_{13} which is processed to the compounds of Formula I by synthetic transformations as outlined in SCHEME 1

SCHEME 6


A19a Y1 = H
A19b Y1 = 6-Me
A19c Y1 = 4-Me
A19d Y1 =4-NO2
A20a Y1= H
A20b Y1 = 6-Me
A20c Y1 = 4-Me
A20d Y1 =4-NO2
1. Mitsunobu
2. Deoxygenation
3. Hydrolysis
A21a Y1= H
A21b Y1 = 6-Me
A21c Y1 = 4-Me
A21d Y1 =4-NO2
A21e Y1 = 4-NH2

SCHEME 6

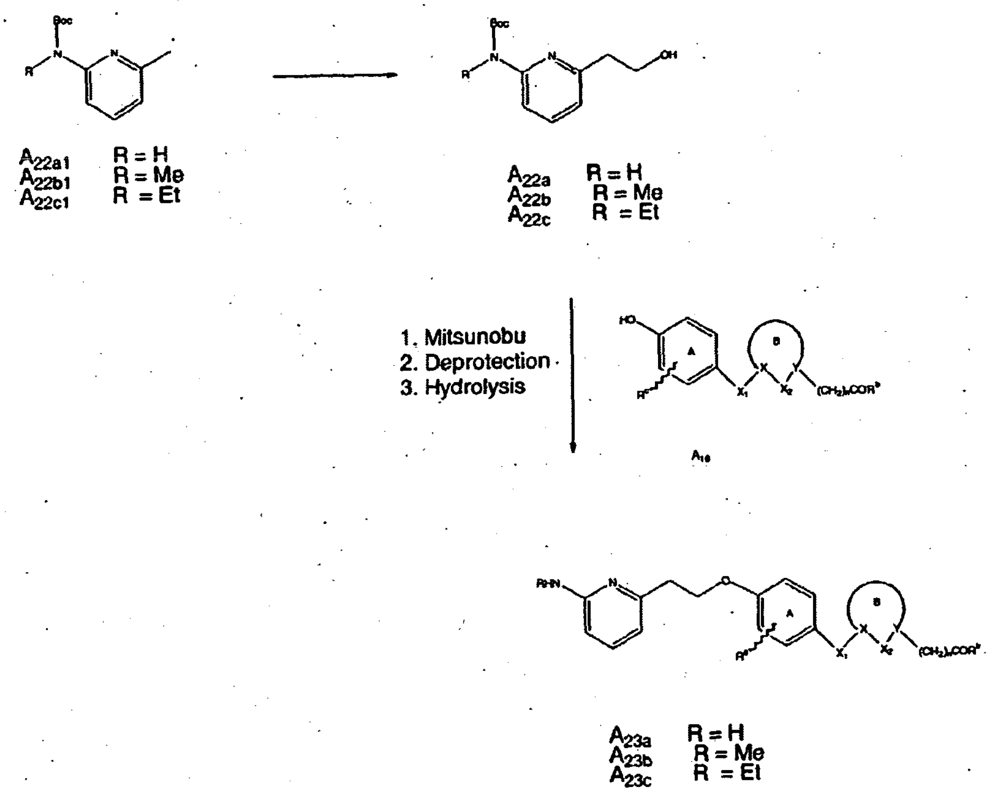
The compounds of FORMULA I, wherein A^1 is substituted pyridyl may be prepared by adopting the general synthetic SCHEME 6. For example, reaction of substituted 2-halopyridine *N*-oxide (such as A_{19a}-A_{19d}) with e. g. 3-aminopropanol gives the intermediates A_{20a}-A_{20d}. This reaction may preferentially be carried out by refluxing the intermediate 2-halopyridine N-oxide (such as 2-chloropyridine N-oxide) in solvents such as tert-butyl alcohol, tert-amyl alcohol in the presence of base (such as sodium bicarbonate, sodium carbonate, potassium carbonate, potassium bicarbonate). The preparative conditions described in WO 99/15508 (PCT US 98/19466) may be used for this transformation.

Coupling of the intermediates A_{20a}-A_{20d} with A_{16} using Mitsunobu reaction gives the compounds containing the ether link. This reaction may preferentially be carried out using triarylphosphine (such as triphenyl-phoshine) and dialkylazodicarboxylate (such as diethyl azodicarboxylate, di-tert-butyl azodicarboxylate, di-iso-propyl azodicarboxylate) in solvents such as DMF, methylene chloride, or THF. N-Deoxygenation of resulting intermediates followed by hydrolysis of the ester gives the compounds (A_{21a}-A_{21d}).

Reduction of the N-oxide bond may be accomplished using e. g., transfer hydrogenation (cyclohexene/Pd on carbon) or ammonium formate and Pd on carbon. The nitro group in A_{21d} may be hydrogenated using Pd on carbon or Pt on carbon as catalysts. This transformation may be carried out using solvents such as methanol, ethanol or THF. The hydrolysis of the ester group may be carried using aqueous base (such as sodium hydroxide, lithium hydroxide or potassium hydroxide) in solvents such as methanol, ethanol and THF.

Compounds of Formula I containing a heterocycle other than pyridyl can also be prepared using the methodology of SCHEME 6. For example reaction of 2-bromopyrimidine or 1-chloroisoquinoline N-oxide with 3-amino-propanol gives the analogous intermediates as obtained in STEP 1 of SCHEME 6. The resulting intermediates could be elaborated as in SCHEME

SCHEME 7

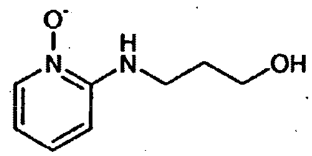

Boc
N
R
N
OH
A22a1 R = H
A22b1 R = Me
A22c1 R = Et
A22a R = H
A22b R = Me
A22c R = Et
1. Mitsunobu
2. Deprotection
3. Hydrolysis
HO
A
B
A18
RHN
A23a R = H
A23b R = Me
A23c R = Et

SCHEME 7

Compounds of FORMULA I containing 6-amino substituents may be prepared as shown in SCHEME 7. The intermediate A_{22b} can be prepared as described in J. Med. Chem 43, 22, 2000. Boc-protected 2-amino-6-picoline (A_{22a1}) or its ethylated derivative (A_{22c1}) are elaborated to A_{22a} and A_{22c} as shown for case A_{22b} in the above publication. The ethylated intermediate A_{22c1} may be prepared from A_{22a1} by alkylation using e. g.; EtI and a base such as potassium carbonate, cesium carbonate. This reaction may preferentially be carried out in polar solvents such as dimethylformamide, or dimethylacetamide. Mitsunobu reaction of A_{16}, gives the compounds containing the phenol ether. Removal of Boc group using e. g., trifluoroacetic acid, in solvents such as dichloromethane, followed by hydrolysis of the ester group as discussed in SCHEME 6 above gives the compounds (A_{23a}-A_{23c}).

EXAMPLE A

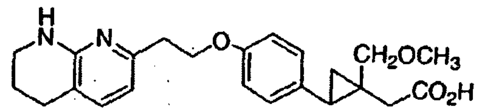
2-[3-hydroxy-1-propylamino]pyridine-*N*-oxide:



A mixture of 2-chloropyridine-N-oxide (16.6 g, 100 mmoles), 3-amino-1-propanol (15.3 ml, 200 mmoles), $NaHCO_3$ (42 g, 0.5 mole), and tert-amyl alcohol (100 ml) was heated to reflux. After 23 hours, the reaction was cooled, diluted with CH_2Cl_2 (300 ml), and filtered to remove insoluble materials. The filtrate was concentrated to afford a brown oil. The oil was dried under vacuum overnight. Ether (100 ml) was added to give a brown solid. The ether was decanted and the solid was washed further with ether/acetonitrile (3/1). The resulting solid was heated at 67°C under vacuum to give the desired product (13.5 g). ^1H NMR was consistent with the proposed structure.

<u>EXAMPLE 33</u>

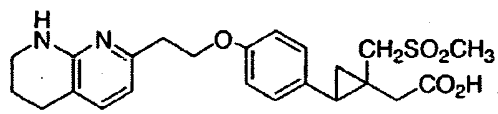
(1-Methoxymethyl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 34

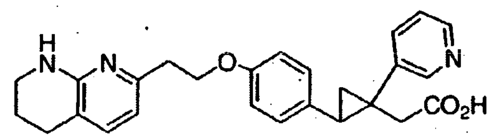
(1-Methanesulfonylmethyl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 35

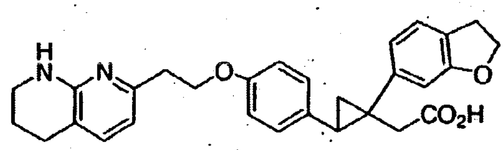
(1-Pyridin-3-yl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 36

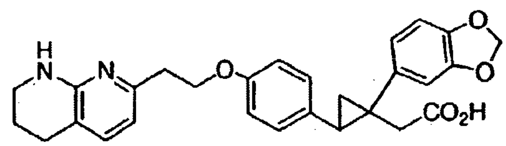
(1-(2,3-Dihydro-benzofuran-6-yl)-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 37

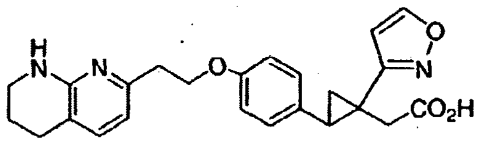
(1-Benzo[1,3]dioxol-5-yl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 38

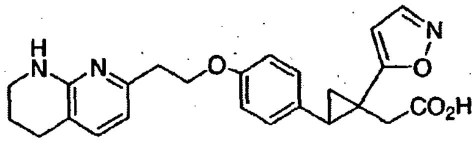
(1-Isoxazol-3-yl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 39

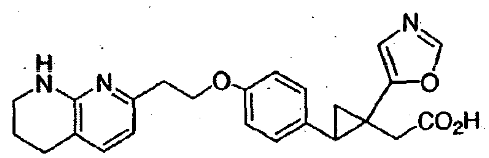
(1-Isoxazol-5-yl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 40

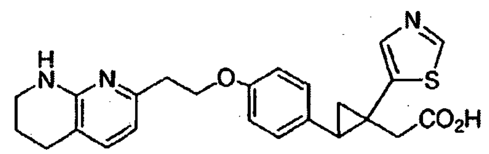
(1-Oxazol-5-yl-2-{4-[2-(5,6,7,8-tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 41

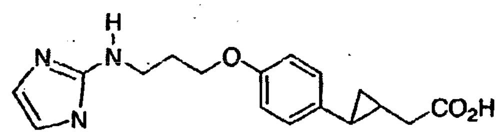
(2-{4-[2-(5,6,7,8-Tetrahydro-[1,8]naphthyridin-2-yl)-ethoxy]-phenyl}-1-thiazol-5-yl-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 8.

EXAMPLE 42

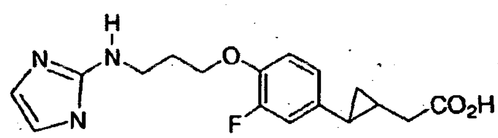
(2-{4-[3-(1-*H*-Imidazol-2-ylamino)-propoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 9.

<u>EXAMPLE 43</u>

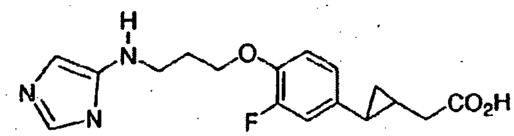
(2-{3-Fluoro-4-[3-(1-*H*-imidazol-2-ylamino)-propoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 9.

EXAMPLE 44

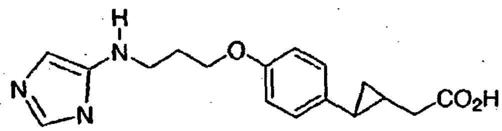
(2-{3-Fluoro-4-[3-(3-*H*-imidazol-4-ylamino)-propoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 9.

EXAMPLE 45

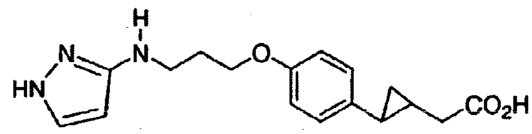
(2-{4-[3-(3-*H*-Imidazol-4-ylamino)-propoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 9.

EXAMPLE 46

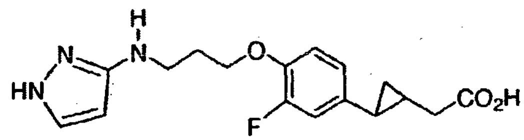
(2-{4-[3-(1-*H*-Pyrazol-3-ylamino)-propoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 9.

EXAMPLE 47

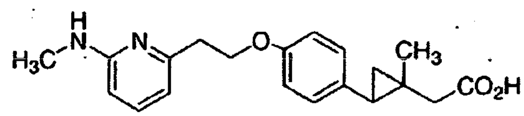
(2-{3-Fluoro-4-[3-(1-*H*-pyrazol-3-ylamino)-propoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 9.

EXAMPLE 48

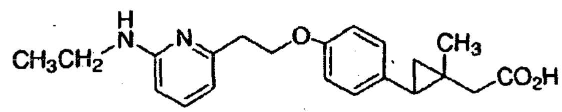
(1-Methyl-2-{4-[2-(6-methylamino-pyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 10.

EXAMPLE 49

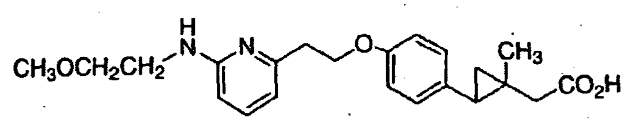
(2-{4-[2-(6-Ethylamino-pyridin-2-yl)-ethoxy]-phenyl}-1-methyl-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described,in SCHEME 10.

EXAMPLE 50

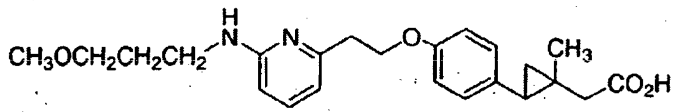
[2-(4-{2-[6-(2-Methoxy-ethylamino)-pyridin-2-yl]-ethoxy}-phenyl)-1-methyl-cyclopropyl]-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 10.

EXAMPLE 51

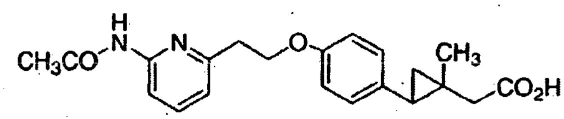
[2-(4-{2-[6-(3-Methoxy-propylamino)-pyridin-2-yl]-ethoxy}-phenyl)-1-methyl-cyclopropyl]-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 10.

EXAMPLE 52

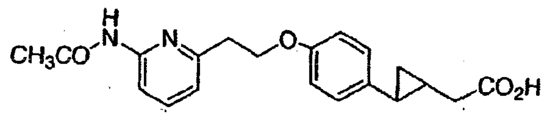
(2-{4-[2-(6-Acetylamino-pyridin-2-yl)-ethoxy]-phenyl}-1-methyl-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 10.

<u>EXAMPLE 53</u>

(2-{4-[2-(6-Acetylamino-pyridin-2-yl)-ethoxy]-phenyl}-cyclopropyl)-acetic acid



The title compound is prepared according to the general procedures described in SCHEME 10.

The activity of the compounds of the present invention was tested in the following assays.

VITRONECTIN ADHESION ASSAY

MATERIALS

Human vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were purified from human placenta as previously described [Pytela et al., Methods in Enzymology, 144:475-489 (1987)]. Human vitronectin was purified from fresh frozen plasma as previously described [Yatohgo et al., Cell Structure and Function, 13:281-292 (1988)]. Biotinylated human vitronectin was prepared by coupling NHS-biotin from Pierce Chemical Company (Rockford, IL) to purified vitronectin as previously described [Charo et al., J. Biol. Chem., 266(3):1415-1421 (1991)]. Assay buffer, OPD substrate tablets, and RIA grade BSA were obtained from Sigma (St. Louis, MO). Anti-biotin antibody was obtained from Sigma (St. Luois, MO). Nalge Nunc-Immuno microtiter plates were obtained from Nalge Company (Rochester, NY).

METHODS

Solid Phase Receptor Assays

This assay was essentially the same as previously reported [Niiya et al., Blood, 70:475-483 (1987)]. The purified human vitronectin receptors $\alpha_v\beta_3$ and $\alpha_v\beta_5$ were diluted from stock solutions to 1.0 µg/mL in Tris-buffered saline containing 1.0 mM Ca^{++}, Mg^{++}, and Mn^{++}, pH 7.4 (TBS^{+++}). The diluted receptors were immediately transferred to Nalge Nunc-Immuno microtiter plates at 100 µL/well (100 ng receptor/well). The plates were sealed and incubated overnight at 4°C to allow the receptors to bind to the wells. All remaining steps were at room temperature. The assay plates were emptied and 200 µL of 1% RIA grade BSA in TBS^{+++} (TBS^{+++}/BSA) were added to block exposed plastic surfaces. Following a 2 hour incubation, the assay plates were washed with TBS^{+++} using a 96 well plate

washer. Logarithmic serial dilution of the test compound and controls were made starting at a stock concentration of 2 mM and using 2 nM biotinylated vitronectin in TBS^{+++}/BSA as the diluent. This premixing of labeled ligand with test (or control) ligand, and subsequent transfer of 50 µL aliquots to the assay plate was carried out with a CETUS Propette robot; the final concentration of the labeled ligand was 1 nM and the highest concentration of test compound was 1.0×10^{-4} M. The competition occurred for two hours after which all wells were washed with a plate washer as before. Affinity purified horseradish peroxidase labeled goat anti-biotin antibody was diluted 1:2000 in TBS^{+++}/BSA and 125 µL was added to each well. After 45 minutes, the plates were washed and incubated with OPD/H_2O_2 substrate in 100 mM/L Citrate buffer, pH 5.0. The plate was read with a microtiter plate reader at a wavelength of 450 nm and when the maximum-binding control wells reached an absorbance of about 1.0, the final A_{450} were recorded for analysis. The data were analyzed using a macro written for use with the EXCEL spreadsheet program. The mean, standard deviation, and %CV were determined for duplicate concentrations. The mean A_{450} values were normalized to the mean of four maximum-binding controls (no competitor added)(B-MAX). The normalized values were subjected to a four parameter curve fit algorithm [Rodbard et al., Int. Atomic Energy Agency, Vienna, pp 469 (1977)], plotted on a semi-log scale, and the computed concentration corresponding to inhibition of 50% of the maximum binding of biotinylated vitronectin (IC_{50}) and corresponding R^2 was reported for those compounds exhibiting greater than 50% inhibition at the highest concentration tested; otherwise the IC_{50} is reported as being greater than the highest concentration tested. β-[[2-[[5-[(aminoiminomethyl)amino]-1-oxopentyl]amino]-1-oxoethyl]amino]-3-pyridinepropanoic acid [US 5,602,155 Example 1] which is a potent $\alpha_v\beta_3$ antagonist (IC_{50} in the range 3-10 nM) was included on each plate as a positive control.

PURIFIED IIb/IIIa RECEPTOR ASSAY

MATERIALS

Human fibrinogen receptor (IIb/IIIa) was purified from outdated platelets. (Pytela, R., Pierschbacher, M.D., Argraves, S., Suzuki, S., and Rouslahti, E. "Arginine-Glycine-Aspartic acid adhesion receptors", Methods in Enzymology 144(1987):475-489.) Human vitronectin was purified from fresh frozen plasma as described in Yatohgo, T., Izumi, M., Kashiwagi, H., and Hayashi, M., "Novel purification of vitronectin from human plasma by heparin affinity chromatography," Cell Structure and Function 13(1988):281-292. Biotinylated human vitronectin was prepared by coupling NHS-biotin from Pierce Chemical Company (Rockford, IL) to purified vitronectin as previously described. (Charo, I.F., Nannizzi, L., Phillips, D.R., Hsu, M.A., Scarborough, R.M., "Inhibition of fibrinogen binding to GP IIb/IIIa by a GP IIIa peptide", J. Biol. Chem. 266(3)(1991): 1415-1421.) Assay buffer, OPD substrate tablets, and RIA grade BSA were obtained from Sigma (St. Louis, MO). Anti-biotin antibody was obtained from Sigma (St. Louis, MO). Nalge Nunc-Immuno microtiter plates were obtained from (Rochester, NY). ADP reagent was obtained from Sigma (St. Louis, MO).

METHODS

Solid Phase Receptor Assays

This assay is essentially the same reported in Niiya, K., Hodson, E., Bader, R., Byers-Ward, V. Koziol, J.A., Plow, E.F. and Ruggeri, Z.M., "Increased surface expression of the membrane glycoprotein IIb/IIIa complex induced by platelet activation: Relationships to the binding of fibrinogen and platelet aggregation", Blood 70(1987):475-483. The purified human fibrinogen receptor (IIb/IIIa) was diluted from stock solutions to 1.0 µg/mL in Tris-buffered saline containing 1.0 mM Ca^{++}, Mg^{++}, and Mn^{++}, pH 7.4 (TBS^{+++}). The diluted receptor was immediately transferred to Nalge Nunc-Immuno microtiter plates at 100 µL/well (100 ng receptor/well). The plates were sealed and incubated overnight at 4°C to allow the receptors to bind to the wells. All remaining steps were at room temperature. The assay plates were emptied and 200 µL of 1% RIA grade BSA in TBS^{+++}

(TBS^{+++}/BSA) were added to block exposed plastic surfaces. Following a 2 hour incubation, the assay plates were washed with TBS^{+++} using a 96 well plate washer. Logarithmic serial dilution of the test compound and controls were made starting at a stock concentration of 2 mM and using 2 nM biotinylated vitronectin in TBS^{+++}/BSA as the diluent. This premixing of labeled ligand with test (or control) ligand, and subsequent transfer of 50 μL aliquots to the assay plate was carried out with a CETUS Propette robot; the final concentration of the labeled ligand was 1 nM and the highest concentration of test compound was 1.0×10^{-4} M. The competition occurred for two hours after which all wells were washed with a plate washer as before. Affinity purified horseradish peroxidase labeled goat anti-biotin antibody was diluted 1:2000 in TBS^{+++}/BSA and 125 μL were added to each well. After 45 minutes, the plates were washed and incubated with ODD/H_2O_2 substrate in 100 mM/L citrate buffer, pH 5.0. The plate was read with a microtiter plate reader at a wavelength of 450 nm and when the maximum-binding control wells reached an absorbance of about 1.0, the final A_{450} were recorded for analysis. The data were analyzed using a macro written for use with the EXCELJ spreadsheet program. The mean, standard deviation, and %CV were determined for duplicate concentrations. The mean A_{450} values were normalized to the mean of four maximum-binding controls (no competitor added)(B-MAX). The normalized values were subjected to a four parameter curve fit algorithm, [Robard et al., Int. Atomic Energy Agency, Vienna, pp 469 (1977)], plotted on a semi-log scale, and the computed concentration corresponding to inhibition of 50% of the maximum binding of biotinylated vitronectin (IC_{50}) and corresponding R^2 was reported for those compounds exhibiting greater than 50% inhibition at the highest concentration tested; otherwise the IC_{50} is reported as being greater than the highest concentration tested. β-[[2-[[5-[(aminoiminomethyl)amino]-1-oxopentyl]amino]-1-oxoethyl]amino]-3-pyridinepropanoic acid [US 5,602,155 Example 1] which is a potent $\alpha_v\beta_3$ antagonist (IC_{50} in the range 3-10 nM) was included on each plate as a positive control.

Human Platelet Rich Plasma Assays

Healthy aspirin free donors were selected from a pool of volunteers. The harvesting of platelet rich plasma and subsequent ADP induced platelet aggregation assays were performed as described in Zucker, M.B., "Platelet Aggregation Measured by the Photometric Method", Methods in Enzymology 169(1989):117-133. Standard venipuncture techniques using a butterfly allowed the withdrawal of 45 mL of whole blood into a 60 mL syringe containing 5 mL of 3.8% trisodium citrate. Following thorough mixing in the syringe, the anti-coagulated whole blood was transferred to a 50 mL conical polyethylene tube. The blood was centrifuged at room temperature for 12 minutes at 200 xg to sediment non-platelet cells. Platelet rich plasma was removed to a polyethylene tube and stored at room temperature until used. Platelet poor plasma was obtained from a second centrifugation of the remaining blood at 2000 xg for 15 minutes. Platelet counts are typically 300,000 to 500,000 per microliter. Platelet rich plasma (0.45 mL) was aliquoted into siliconized cuvettes and stirred (1100 rpm) at 37°C for 1 minute prior to adding 50 uL of pre-diluted test compound. After 1 minute of mixing, aggregation was initiated by the addition of 50 uL of 200 uM ADP. Aggregation was recorded for 3 minutes in a Payton dual channel aggregometer (Payton Scientific, Buffalo, NY). The percent inhibition of maximal response (saline control) for a series of test compound dilutions was used to determine a dose response curve. All compounds were tested in duplicate and the concentration of half-maximal inhibition (IC_{50}) was calculated graphically from the dose response curve for those compounds which exhibited 50% or greater inhibition at the highest concentration tested; otherwise, the IC_{50} is reported as being greater than the highest concentration tested.